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Docket No. CL2180 US NA

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REMARKS/ARGUMENTS

Status of the Application

In the Non-Final Office Action mailed July 14, 2006, claims 1-3 and 8 were rejected, claims 3 and 8 were objected to, and claims 4-7 were withdrawn from consideration. In the present response, claims 3 and 8 were amended to remove the term "further" from the claims and to correct grammatical errors. Claim 3 was further amended to remove non-elected subject matter.

Thus, claims 1-3 and 8 are pending. No new matter was added.

Claim Objections

Claim 3 was objected to as encompassing non-elected subject matter. Applicants respectfully submit that the present amendment to claim 3 obviates the objection.

Claims 3 and 8 were objected to for the recitation of "further" in the term "further comprising". Applicants have deleted the word "further" from both claims.

Rejection Under 35 U.S.C. § 112, 2nd Paragraph

Claim 8 was rejected under 35 U.S.C. § 112, 2nd Paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner asserted that the recitation of the terms "a 1.6 long GI promoter" and "a 1.5 long GI promoter" in claim 8 was indefinite. Applicants respectfully traverse this rejection.

The terms "a 1.6 long GI promoter" and "a 1.5 long GI promoter" are described in the specification at page 32, line 31 – page 33, line 24 and in SEQ ID NOs: 65-68. By referencing SEQ ID NOs: 65-68, the exact sequences of these promoters are available to one skilled in the art in possession of Applicants' specification. Thus, the terms, used as descriptors of these promoters, are definite.

Rejections Under 35 U.S.C. § 112, 1st Paragraph

Claims 1-3 and 8 were rejected under 35 U.S.C. § 112, 1st Paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the

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time the application was filed, had possession of the claimed invention. Applicants respectfully traverse these rejections.

The examiner states that each genus of the group of genes involved [i.e. a.), b.) c.), d.), i.), etc.] is described in the specification by only a single representative species, thus, failing to describe any other representative species. The examiner states the specification fails to describe a method of achieving disruption (of *PTS* or *arcA*), up regulation (of *glk* and *galP*), or down regulation (of *gapA*) by methods other than phage transduction, promoter replacement (with strong promoter), and replacing an ATG start codon for down regulation, respectively.

Applicants respectfully submit that an *E. coli* strain having the claimed disruptions, up-regulations, and down-regulations to endogenous genes is well-described in the working examples and throughout the specification. Each *E. coli* gene is well-described in the specification (see page 12, line 21 – page 20, line 15). Applicants have provided several biological deposits (see page 11, lines 8-36), detailed working examples are provided, and multiple methods of achieving disruption (distinct from down regulating), up regulation, and down regulation, which are applicable regardless of gene target, are similarly well-described (see, e.g., page 35, line 12 – page 36, line 26). Thus, Applicants' specification provides a detailed description of the disrupted, up-regulated, and down-regulated endogenous *E. coli* genes, has pointed the skilled person to a number of specific sequences with structural features for each gene, and has fully described methods of gene manipulation useful in practicing the present invention. Applicants thus respectfully submit that their specification has put the skilled person on notice that Applicants were in possession of the claimed invention at the time of filing.

Claims 1-3 and 8 were rejected under 35 U.S.C. § 112, 1st Paragraph, because the specification, while being enabling for an *E. coli* strain KLpts7 comprising a) a disrupted endogenous phosphoenolpyruvate-glucose phosphotransferase system (operon) by using P1 phage transduction of kanamycin antibiotic resistance marker which places the operon genes comprising *ptsH*, *ptsI*, and *crr* with the kanamycin resistance marker; b) an up-regulated endogenous *galP* gene, which is under a strong *trc* promoter, encoding active galactose-proton symporter; c) an up-regulated endogenous *glk* gene, which is under a strong *trc*

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promoter, encoding active glucokinase; d) a down-regulated endogenous *gapA* gene encoding active glyceraldehyde-3-phosphate dehydrogenase by replacing the ATG start codon with GTG or TTG; and a disrupted endogenous *arcA* gene by using pKD3 gene knockout system for preventing expression of active aerobic respiration control protein and further comprising one plasmid comprising a first operon comprising genes encoding glycerol-3-phosphate dehydrogenase and glycerol-3-phosphatase, a second operon further comprising a 1.6 long GI promoter controlling genes encoding dehydratase and a first subunit of dehydratase reactivation factor, having the sequence SEQ ID NO:68, does not reasonably provide enablement for any *E. coli* strain comprising a) any disrupted endogenous phosphoenolpyruvate-glucose phosphotransferase system (operon); b) up-regulation of any endogenous *galP* gene; c) up-regulation of any endogenous *glk* gene; d) down-regulation of any endogenous *gapA* gene; and disruption of any endogenous *arcA* gene and further comprising any glycerol-3-phosphate dehydrogenase gene and any glycerol-3-phosphatase gene. Applicants respectfully traverse these rejections.

The touchstone of the enablement requirement is whether the skilled person can make and use the invention without undue experimentation. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). Applicants submit that the skilled person, in possession of the present application describing *E. coli* strains having the claimed gene disruptions, up-regulations, and down-regulations, in conjunction with well-known protocols of molecular biology (see, e.g., page 42, lines 21-35, of the Applicants' specification), would have no difficulty in practicing the invention without undue experimentation.

The Wands factors are: (a) the quantity of experimentation necessary, (b) the amount of direction or guidance presented, (c) the presence or absence of a working example, (d) the nature of invention, (e) the state of prior art, (f) the relative skill of those in the art, (g) the predictability or unpredictability of the art, and (h) the breadth of the claim. In relation thereto, Applicants respectfully assert the following: Factor (a), the amount of experimentation needed to practice the invention is reasonable and commensurate with the art. Factor (b), Applicants' specification provides description of the disrupted, up-regulated, and/or down-regulated *E. coli* genes in claims 1-3 and 8 (see page 12, line 21 – page 20, line 15). Several specific *E. coli*

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strains are mentioned (see page 32, lines 2-4). The specification also provides description of methods for *E. coli* gene disruption, up-regulation, and down-regulation, which as noted above are applicable regardless of the gene target (see, e.g., page 35, line 12 – page 36, line 26). One skilled in the art should be familiar with the methods for *E. coli* gene disruption, up-regulation, and down-regulation described in the Applicants' specification. The Examiner's discussion of amino acid structural and functional properties is outside the scope of the claims and is thus irrelevant to whether Applicants have provided sufficient guidance as to practicing the claimed invention. Factor (c), Applicants have provided working examples demonstrating the production of *E. coli* containing the claimed gene disruptions, up-regulations, down-regulations. Biological deposits and gene sequences are also provided. Factor (d), the invention relates to gene mutations in *E. coli*. Such art requires some laboratory testing for even routine techniques. Therefore, one skilled in the art would expect some testing, screening, and trial and error to implement the present invention outside the working examples. However, the information presented in the instant application is sufficient to enable one skilled in the art to implement the gene disruptions, up-regulations, and down-regulations needed to practice the invention. Factor (e), as stated in the background of the invention (see page 1, line 22 – page 3, line 27), biological production of 1,3-propanediol via fermentation of glycerol is well-known. *E. coli* containing the genes responsible for conversion of glycerol to 1,3-propanediol in *Klebsiella pneumonia* and *Citrobacter freundii* are also known. However, biological methods of 1,3-propanediol production, in addition to well-known chemical production methods, are unsuitable for industrial scale 1,3-propanediol production because they are energy intensive; require the use of an expensive starting material, glycerol; and have low yields (see page 3, lines 18-27; page 5, line 27-33). Applicants have successfully developed an *E. coli* that utilizes a low cost carbon substrate (e.g., glucose) to produce 1,3-propanediol at high yields. The methods for gene disruption, up-regulation, and down-regulation needed to practice Applicants' invention are well-known in the art (see page 35, line 12 – page 36, line 26). Factor (f), this invention is related to the biotechnical arts in an extremely well-known organism having disruptions, up-regulations, down-regulations of well-known genes, and the skill level of the artisan is very high. The

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skilled artisan is therefore very familiar with *E. coli* strains and well versed in many methods and techniques of gene manipulation. Factor (g), the biotechnical art is an unpredictable art; it is not reasonable for an applicant to provide a cookbook recipe of how to practice the invention. Rather, Applicants have depended on the skill and experience of the artisan to implement the invention using gene disruption, up-regulation, and down-regulation methods of their choosing. It is expected that the artisan would be aware of successful methods of *E. coli* gene mutations and therefore be capable of implementing the described genetic manipulations in *E. coli*. Factor (h), the breadth of the claim is reasonable given the vast improvement and the ability of skilled artisans to implement the invention in *E. coli*. It would be unfair to the Applicants to limit their invention to the working examples as the Applicants' specification has provided enough description to allow others in the art to use the present invention with any *E. coli* having the claimed disruptions, up-regulations, and down-regulations.

Rejections Under 35 U.S.C. § 103

Claims 1-3 and 8 were rejected under 35 U.S.C. § 103(a) as being obvious over Baez *et al.* (Biotechnol. Bioeng. 73:530-35 (2001)) in view of Seta *et al.* (J. Bacteriol. 179:5218-21 (1997)) in further view of Iuchi *et al.* (Proc. Natl. Acad. Sci. USA 85:1888-92 (1988)) in further view of Emptage *et al.* (WO01/12833) in further view of Payne *et al.* (Published U.S. Patent Application No. 2005/0147968). Applicants respectfully traverse these rejections.

Claim 1 is drawn to an *E. coli* strain comprising a disruption in endogenous phosphoenolpyruvate-glucose phosphotransferase (PTS) system proteins, an up-regulated *galP* gene encoding active galactose-proton symporter, an up-regulated endogenous *glk* gene, and a down-regulated endogenous *gapA* gene. In rejecting the claims, the Examiner asserts that Baez *et al.* relates to phosphoenolpyruvate-glucose phosphotransferase system proteins (PTS), the *galP* gene, and the *glk* gene; Seta *et al.* relates to the *gapA* gene; Iuchi *et al.* relates to the *arcA* gene; Emptage *et al.* relates to a process for the biological production of 1,3-propanediol; and Payne *et al.* relates to plasmids (e.g., SEQ ID NO:68).

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With respect to claim 1, the disclosures of *luchi et al.* and *Payne et al.* do not apply and thus will not be addressed herein. *Emptage et al.* is a general reference related to biological production of 1,3-propanediol with no specific relevance to claim 1 and will also not be addressed herein.

Applicants thus respectfully traverse the rejection of claim 1 under 35 U.S.C. § 103(a) as being obvious over *Baez et al.* in view of *Seta et al.* *Baez et al.* do not teach an *E. coli* strain comprising reduced expression *gapA*; conversely, *Seta et al.* do not teach an *E. coli* strain with alteration to *PEP*, *galP*, or *glk*. Neither *Baez et al.* nor *Seta et al.* teach or suggest the utility of their respective disclosures, alone or in combination, for the production of 1,3-propanediol. Thus, no motivation is provided to combine their teaching.

Further, *Baez et al.* do not teach a strain comprising *glk*, wherein the activity is higher than the parental strain. *Baez et al.* disclose a PTS⁺ glucose⁺ strain (PB103, taken to be wild type), a PTS⁻ glucose⁻ strain (NF6, derived from PB103), and a PTS⁻ glucose⁺ strain (NF9, derived from NF6). *Baez et al.* report the glucokinase specific activity of strains PB103, NF6, and NF9 to be 134±20, 123±17, and 119±21 $\mu\text{mol/mg/min}$ (see Table II, p. 533). Within the standard deviation (S.D.) of the measurements, the values are identical. Thus, while *Baez et al.* propose the necessity of *glk* expression for glucose consumption in an *E. coli* strain comprising a non-functional or deleted PTS system, they do not teach increased *glk* expression and do not suggest that benefit could be derived from such increased expression.

As claim 8 includes in parts a) – d) the limitations of claim 1, the arguments stated above are equally applicable to claim 8.

Applicants thus respectfully submit that the claims are nonobvious over the cited references.

SUMMARY

In view of the foregoing remarks, Applicants submit that this application is in condition for allowance. In order to expedite disposition of this case, the Examiner is invited to contact either of Applicants' representatives at the telephone numbers listed below to resolve any remaining issues. Should there be a fee due which is not

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accounted for, please charge such fee to Deposit Account No. 04-1928 (E.I. du Pont de Nemours and Company).

Respectfully submitted,

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Dated: December 14, 2006